Calorie restriction protects neural stem cells from age-related deficits in the subventricular zone

Apple DM, Mahesula S, Fonseca RS, Zhu C, Kokovay E. Calorie restriction protects neural stem cells from age-related deficits in the subventricular zone. Aging (Albany NY). 2019;11(1):115-26.

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Calorie restriction protects neural stem cells from age-related deficits in the subventricular zone

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I Introduction

Assumptions and models the authors reference - Adult Neurogenesis in the SVZ

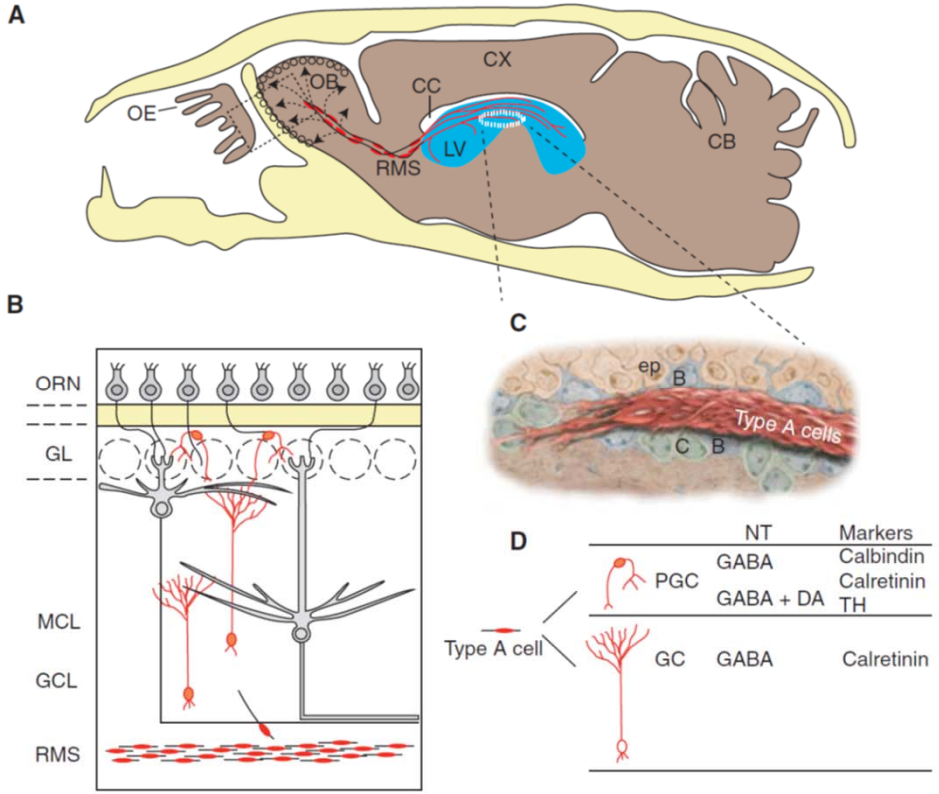


Figure 1. Overview of adult mouse olfactory bulb (OB) neurogenesis from the ventricular–subventricular zone (V-SVZ). (A) Sagittal section through mouse head (calvarium is yellow). Neuroblasts (type A cells) born in the V-SVZ of the lateral ventricle (blue) migrate through a network of paths (red) into the rostral migratory stream (RMS), which enters the OB. Cells then leave the RMS (arrows, dashed lines) and migrate radially into the OB. Boxed area is shown enlarged in B. (B) Neuronal layers of OB. Migratory cells depart the RMS and differentiate into granule cells (GC) or periglomerular cells (PGC), which reside in the granule cell layer (GCL) and glomerular layer (GL), respectively (type A cells and differentiated interneurons are red). ORNs (small gray cells) in the olfactory epithelium (OE) project to the GL. The main projection neurons of the OB (mitral cells and tufted cells) are in gray. (C) Artists' rendition of a chain of migratory type A cells. These chains are ensheathed by glial cells (type B cells, blue) and are associated with clusters of transit-amplifying cells (type Ccells, green). (D) Diversity ofOBinterneurons. Type Acells differentiate into either PGCs or GCs, which can be distinguished by morphology, neurotransmitter (NT) phenotype, and markers. CC, Corpus callosum; Cx, cortex; CB, cerebellum; ORN, olfactory receptor neuron; MCL, mitral cell layer; ep, ependymal cell; TH, tyrosine hydroxylase.

The rodent CNS harbours neural stem cell pools, primarily in two neurogenic niches:

The subgranular zone of the Dentate Gyrus

The Subventricular zone

These Neural stem cells can give rise to neuroblasts -> which can migrate from the SVZ to the olfactory bulbs through the rostral migratory stream.

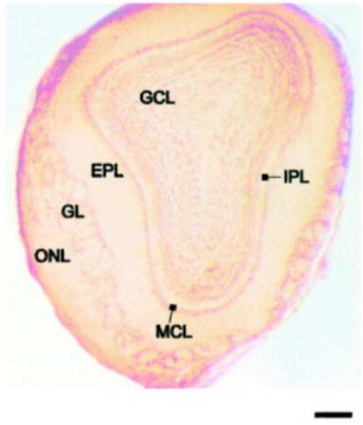


Figure taken from: Lim DA, Alvarez-Buylla A. The Adult Ventricular-Subventricular Zone (V-SVZ) and Olfactory Bulb (OB) Neurogenesis. Cold Spring Harb Perspect Biol. 2016;8(5).

The neurogenic niche in SVZ is tightly regulated

The authors focus on the positional remodeling and progressive activation of Microglial cells -> Contributing to Age-Associated Reductions in Neurogenesis

(further reading: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4817564/pdf/scd.2015.0319.pdf)

And the negative cross talk between inflammation and senescent cells.

Furthermore, they suggest age related dysfunction of the underlying vascular plexus might disrupt neurogenesis

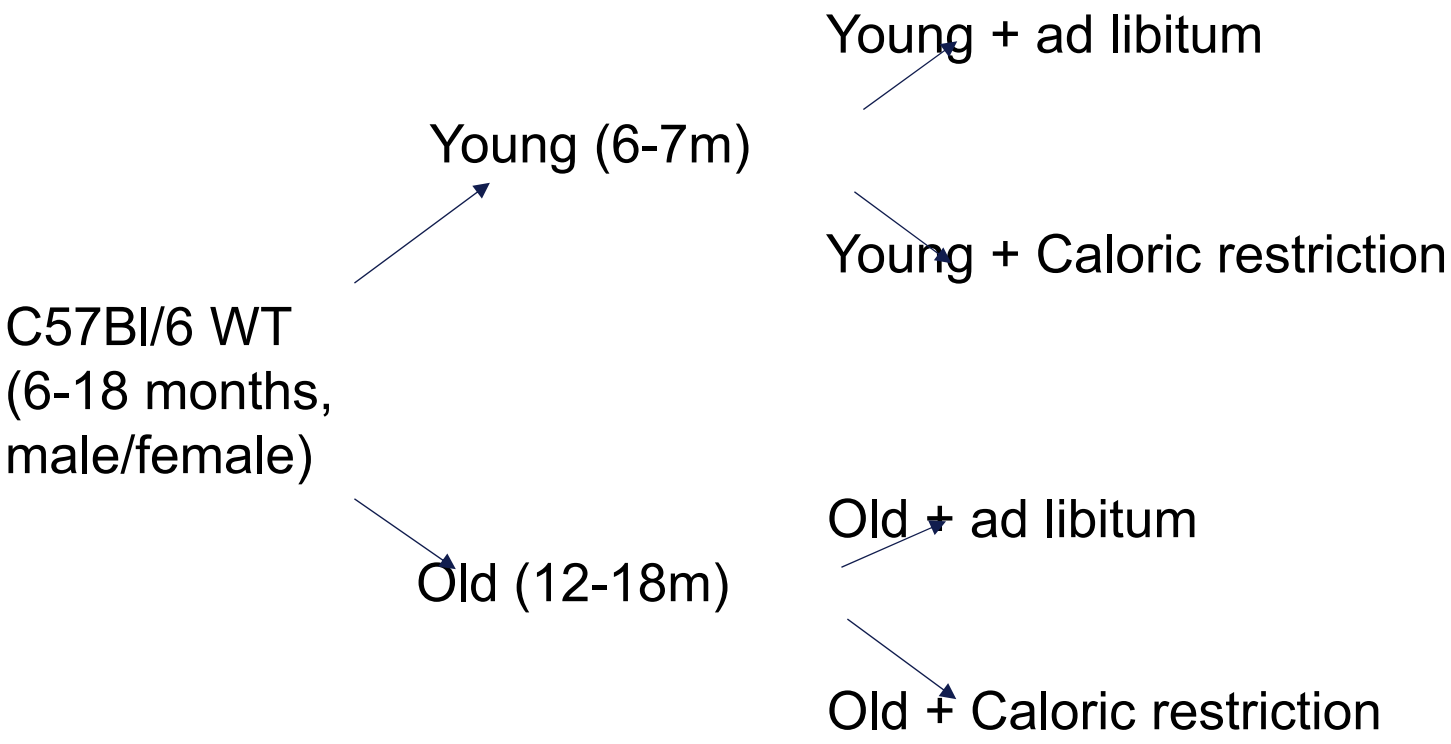
Referenced beneficial effects of Calorie restriction

- Increase in mean and maximum lifespan
- Enhanced insulin sensitivity, BMI reduction
- Cardiovascular risk reduction
- Protective against neurodegeneration

-> The authors suggest that amelioration of „Inflammaging“ and the protection of vascular integrity via caloric restriction might benefit neurogenesis capactiy in the aging brain.

II Methods

General study design



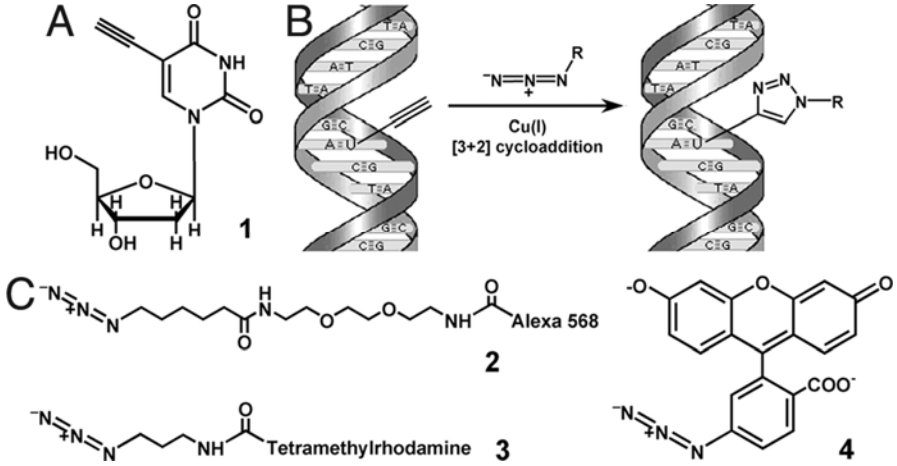
Caloric restriction regime: Initiation at 14 weeks of age (for all?)
Restriction to 40% of free feeding weight
by 16 weeks (10% restriction at 14 weeks, 25% restriction at 15 weeks, and 40% restriction at 16 weeks) of age.
Mice maintained until 6 OR 12-18 months at 40% reduction.

Quantification of Neurogenesis

- **Basic idea:**

In vitro or in vivo application of nucleoside analogs, such as **5-bromo-2'-deoxyuridine BrdU**
-> incorporation into proliferating cells

-> Detection of BrdU using specific antibodies and fluorophores



- (*A*) **5-ethynyl-2′-deoxyuridine EdU** is incorporated into DNA of mitotic cells
- (B) “Click reaction“ terminal alkyne group of EdU conjugateds to organic azide R
- (C) R can be can be any fluorophore, hapten, electron-dense particle, quantum dot, etc. (here:

Paradigm in this study:

Sacrifice at 6 or 12-15m
-> **EdU 2h before** Sacrification
-> „Snapshot of proliferation“
-> **4/5 daily** injections with **BrdU** -> two weeks after last i.p. -> Sacrifice

Approach of establishing mechanisms of the putative beneficial effect of caloric restriction on neurogenesis

Importantly cell proliferation as indicated By EdU, BrdU is not adressing cell fate!
-> Anti-DCX and Anti-GFAP stainings to identify Neuroblasts

- **Brain vasculature and Inflammation**

-> Immunohistochemistry:

Anti Laminin-AB: Vasculature

Anti IBA-1/Anti CD-68: Microglia/Macrophage Markers, both upregulated during inflammation

-> PCR: mRNA: IL-1b, IL-6

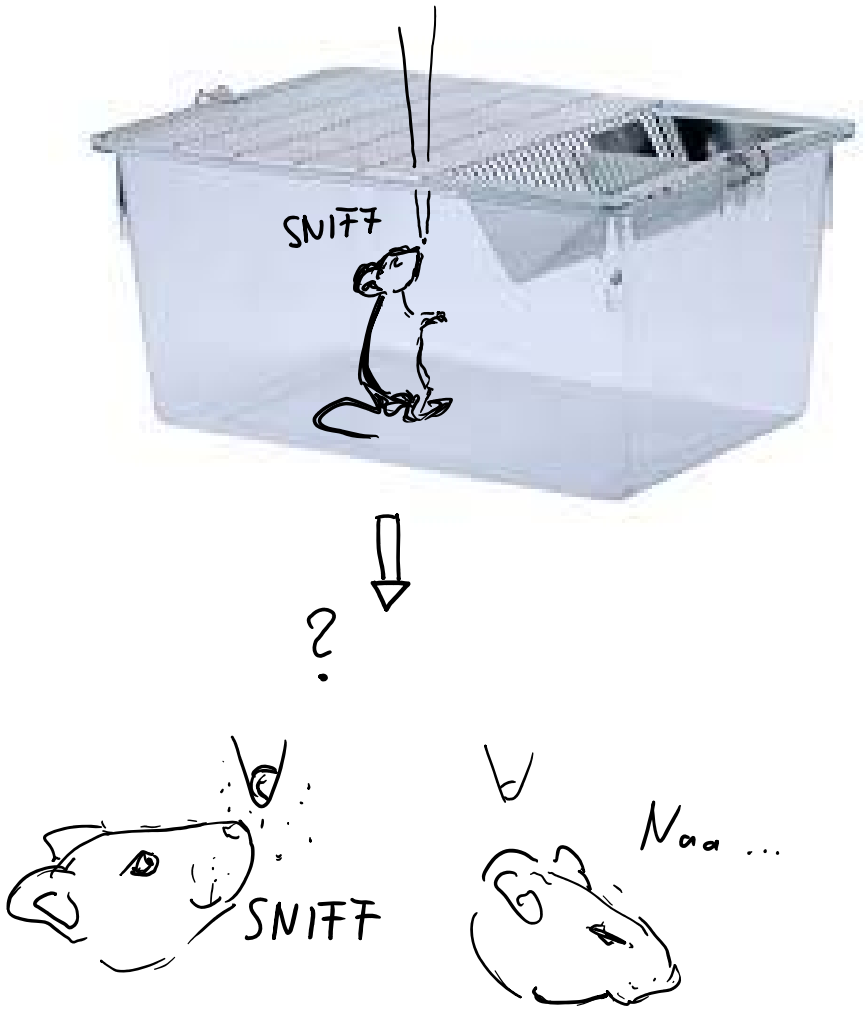
Senescent cells:

Calbiochem Detection set.

-> Count of Beta-galactosidase + cells in the SVZ

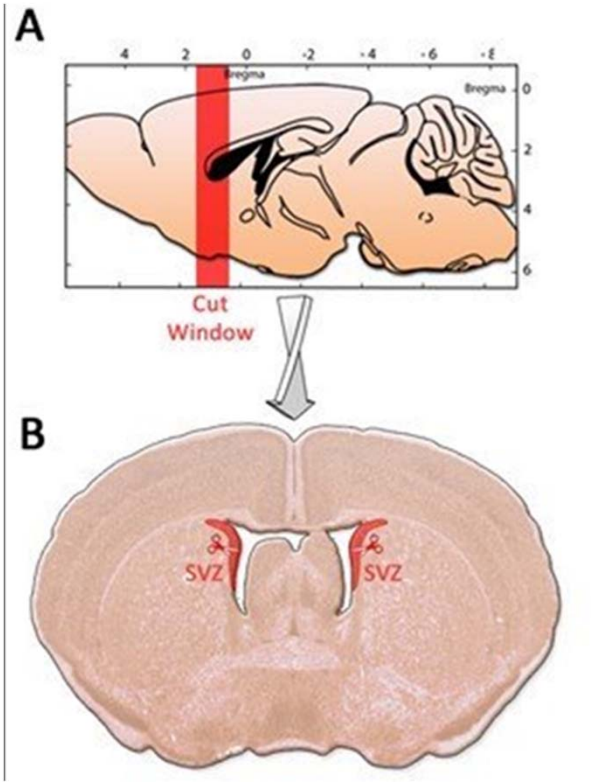
The activity of the lysosomal enzyme β-galactosidase detectable at pH 6.0 is regarded as a marker for senescent cell.

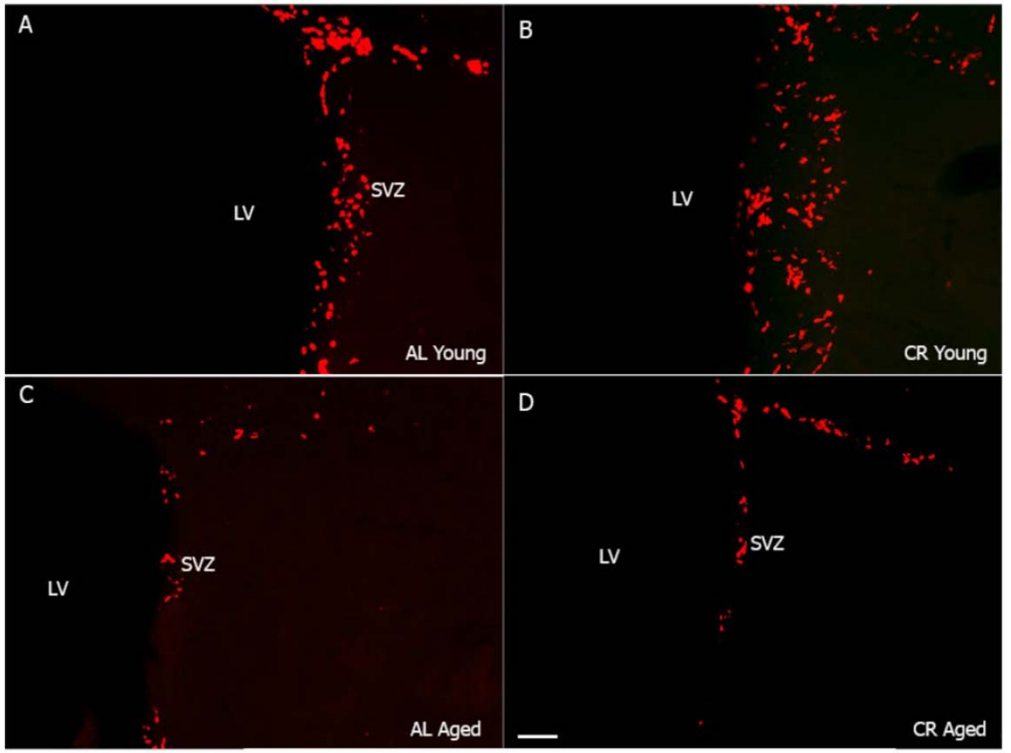
Behavioral analysis



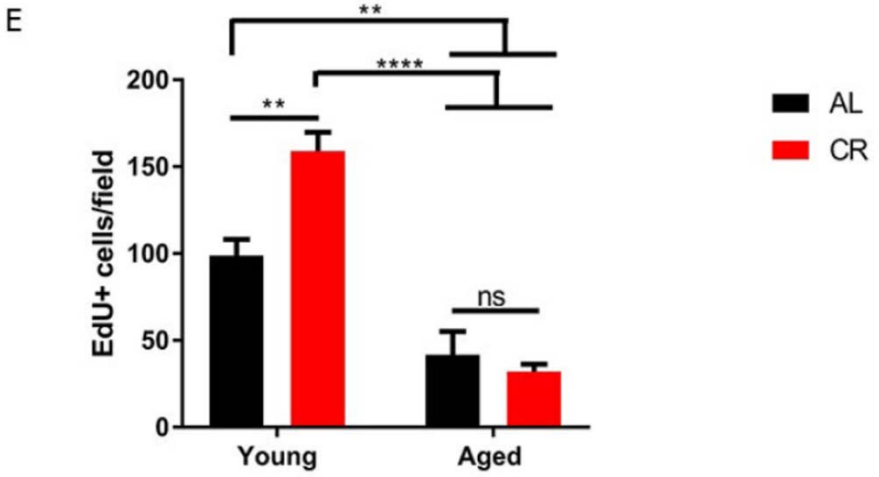
- Novel non food oder presented for a 5min period
- Rearing and sniffing are quantified
- Removal of odor
- Reintroduction of same odor at 30, 60, 120, 180 time intervals.
- Significant decrease in investigation time during the second presentation indicates that mice were able to recognize an odor that had been presented previously

III Results

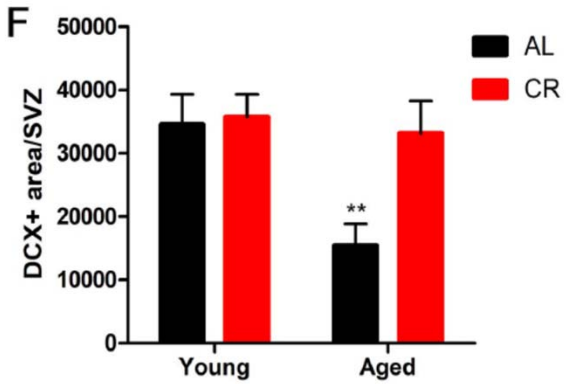


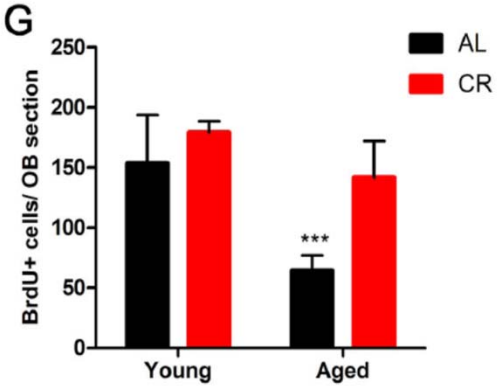


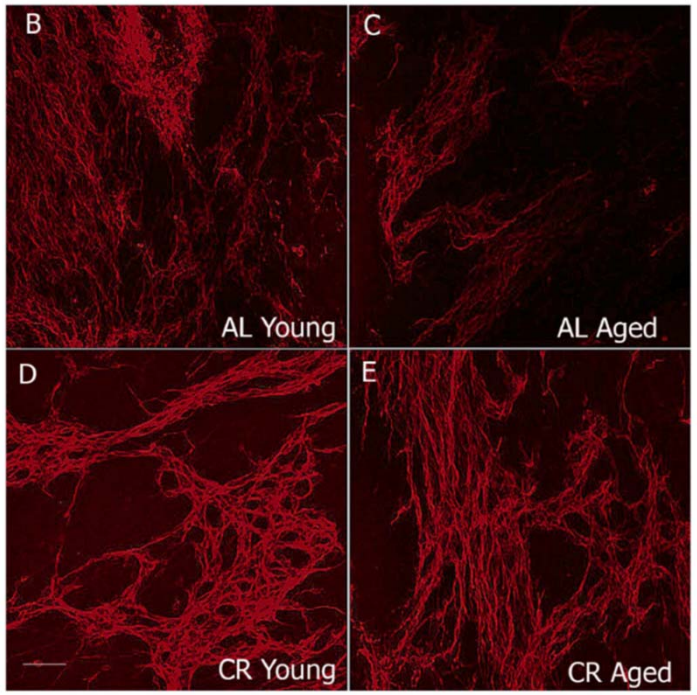
- Transient increase in cell proliferation in the SVZ in young but not old CR mice (roughly 40% difference)



Upper right figure from: https://www.researchgate.net/publication/308754513_Primary_Culture_of_SVZ-derived_Progenitors_Grown_as_Neurospheres (last accessed on 15.03.2020)





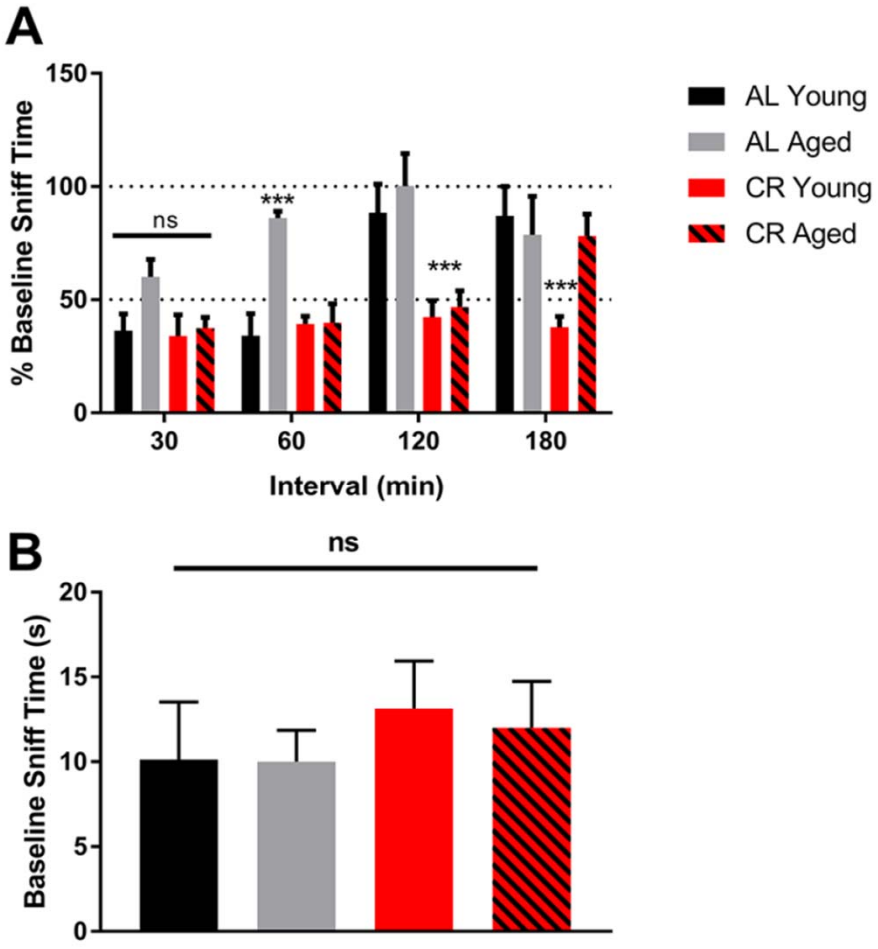


- Arround 2-fold increase of Neuroblasts in CR aged mice in comparison to age matched AL mice

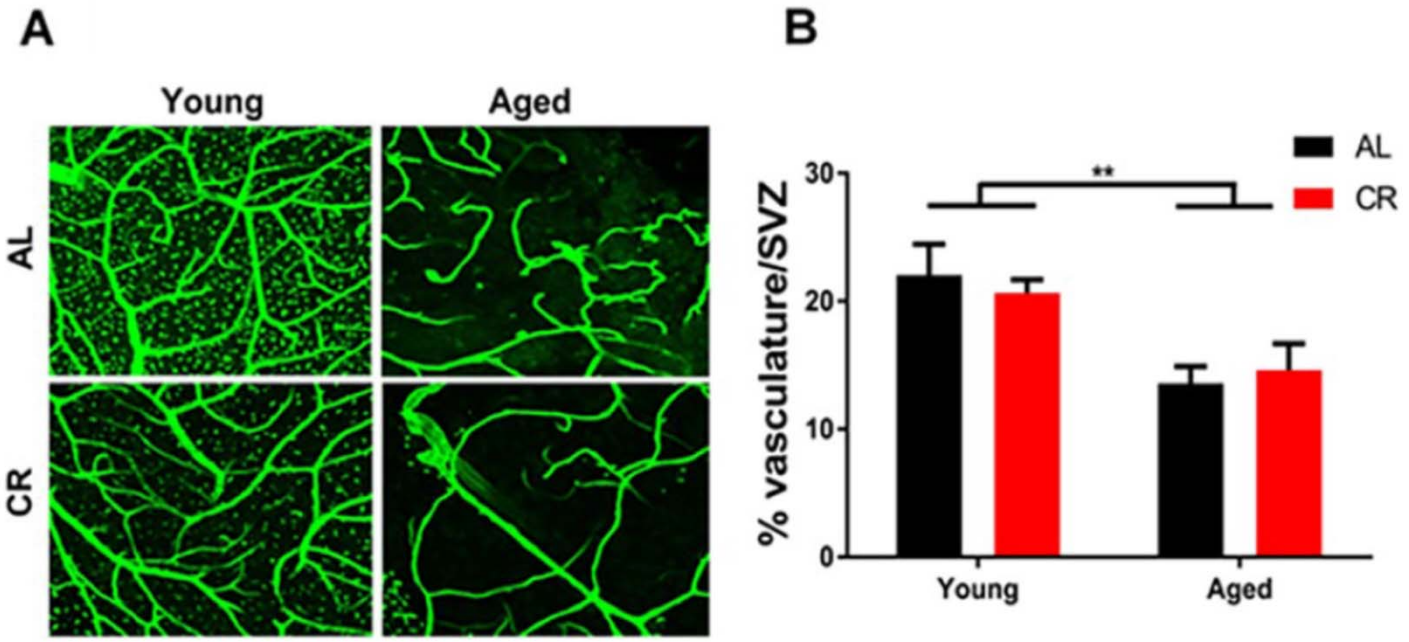
Arround 2-fold increase of BrdU in the Olfactory bulbs in CR aged mice

(BrdU i.p. for 5 days,
2 weeks after last injection
Sacrification)

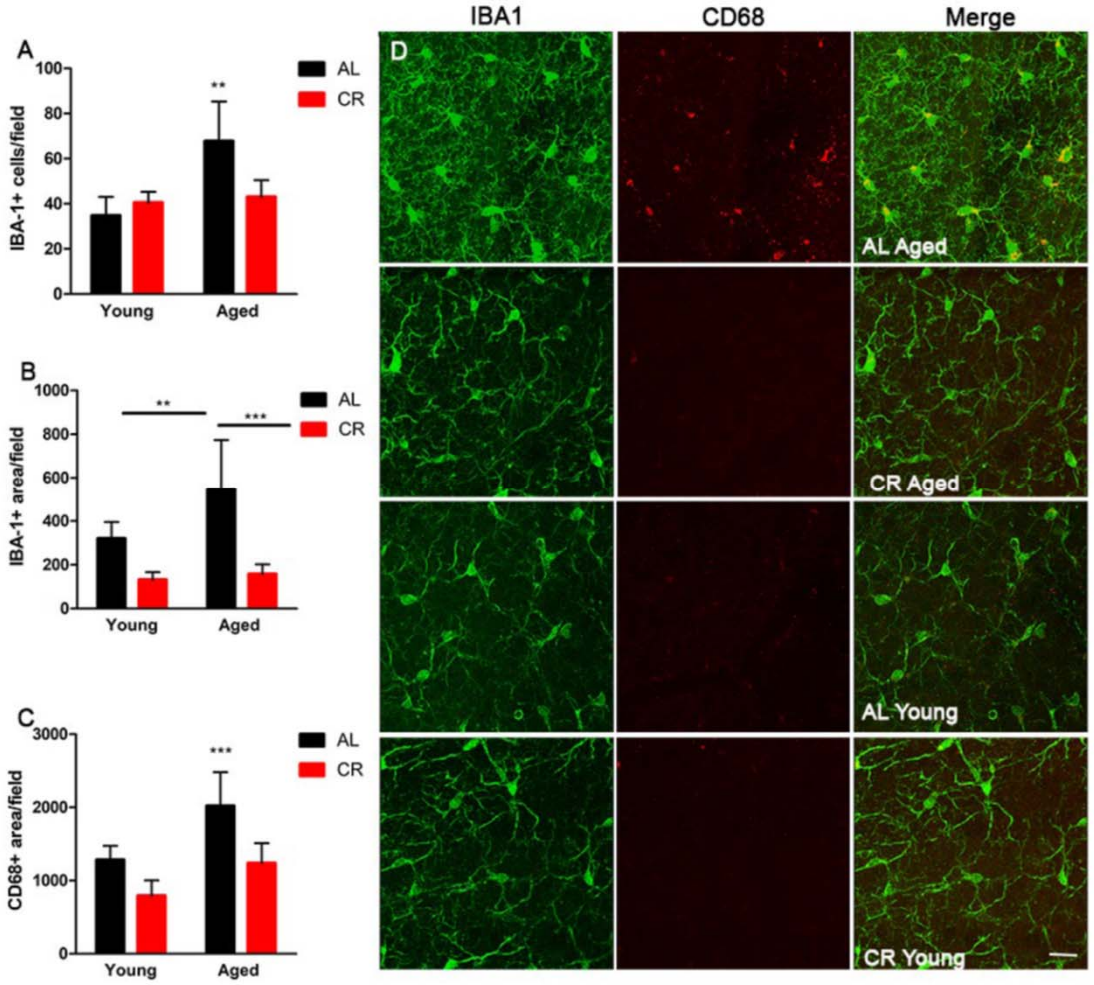
Olfactory memory



- No differences in Baseline Sniff time
- **60 min interval** between odor presentations:
  Aged AL mice almost reached 100% of the baseline sniff time.
- **120 min interaval**
  CR mice of both age groups showed reduced sniff times
- **180 min interval**
  Only CR young show sig. reduced sniff times



- Vascular density declines with age in the SVZ and is not rescued by caloric restriction



Microglia activation is mitigated by calorie restriction

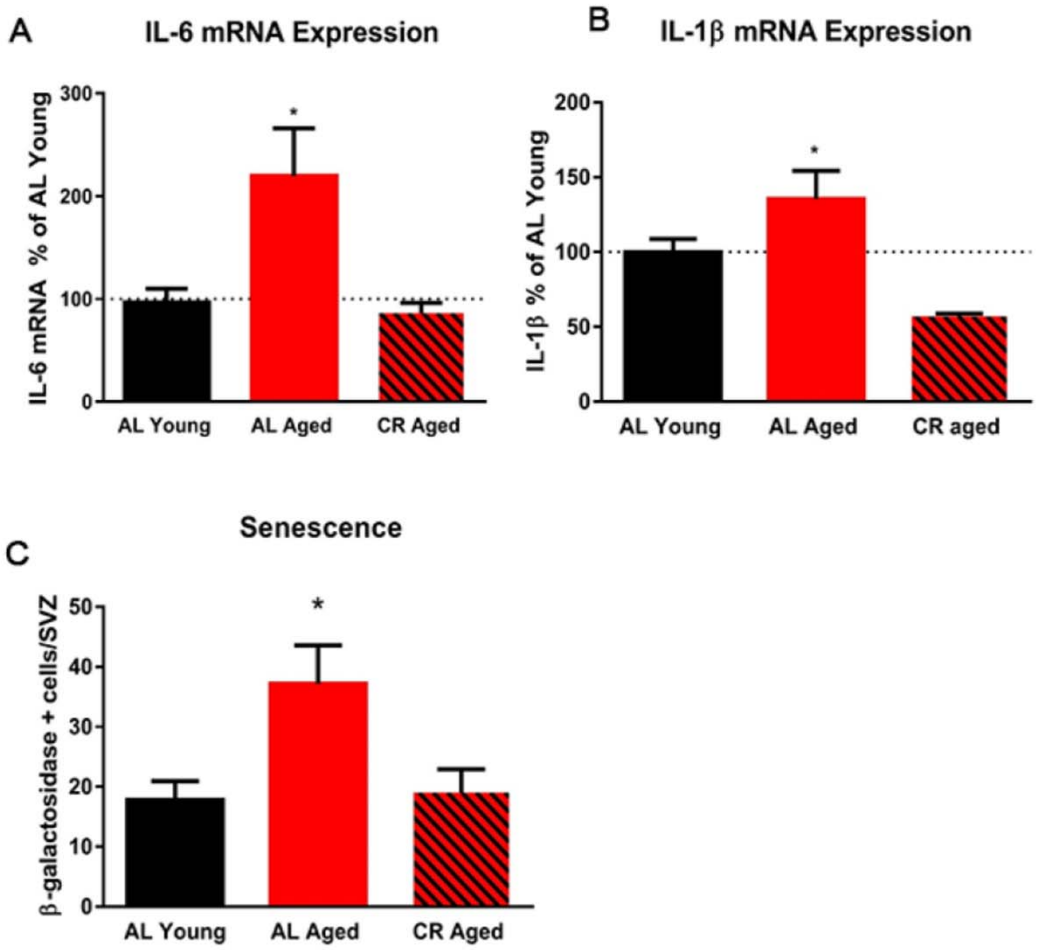
More Iba-1and CD68 cells in AL aged mice but not in CR aged mice.

Iba-1 cells cover more area in the aged AL mice.

-> Suggestive of amoeboid morphology

-> Inflammatory phenotype

Calorie restriction abrogates markers of inflammation in the SVZ



IV Discussion

Conclusions the authors draw

- Calorie restriction initiated in early adulthood prevented age related decline in neurogenesis (More DCX+ cells in the SVZ and BrdU+ cells in OB)
  BUT: Increase in transient proliferation capacity in the SVZ only in young! -> Conflicting findings

- Authors hypotheses/speculations:

  Calorie restriction increased neuroblast survival, but not baseline proliferation?

  Calorie restriction shifted cell fate

“ Together, our data show that **calorie restriction preserves** the **ability of neural stem cells in aged mice to differentiate into neurons in vivo and survive after integration into the olfactory tissue**.”

- This proposed effect on neurogenesis is reflected in enhanced olfactory memory
- The beneficial effects might be related to a decrease of a low level inflammatory status during ageing and less accumulation of senecent cells
- Further studies to establish these links mechanistically are encouraged

Possbile issues – matters of debate

- The behavioral model is close to none replicable with the given information (Housing, Light Dark Cycle, Habituation periods, further environomental enrichment…)

- Focus on neuronal cell fate and one neurogenic niche

- Discrepancies, conflicting evidence regarding the effects of caloric restriction on neurogenesis in the dentate gyrus

Translational relevance?

- The relevance of adult neurogenesis in humans is still a matter of debate!

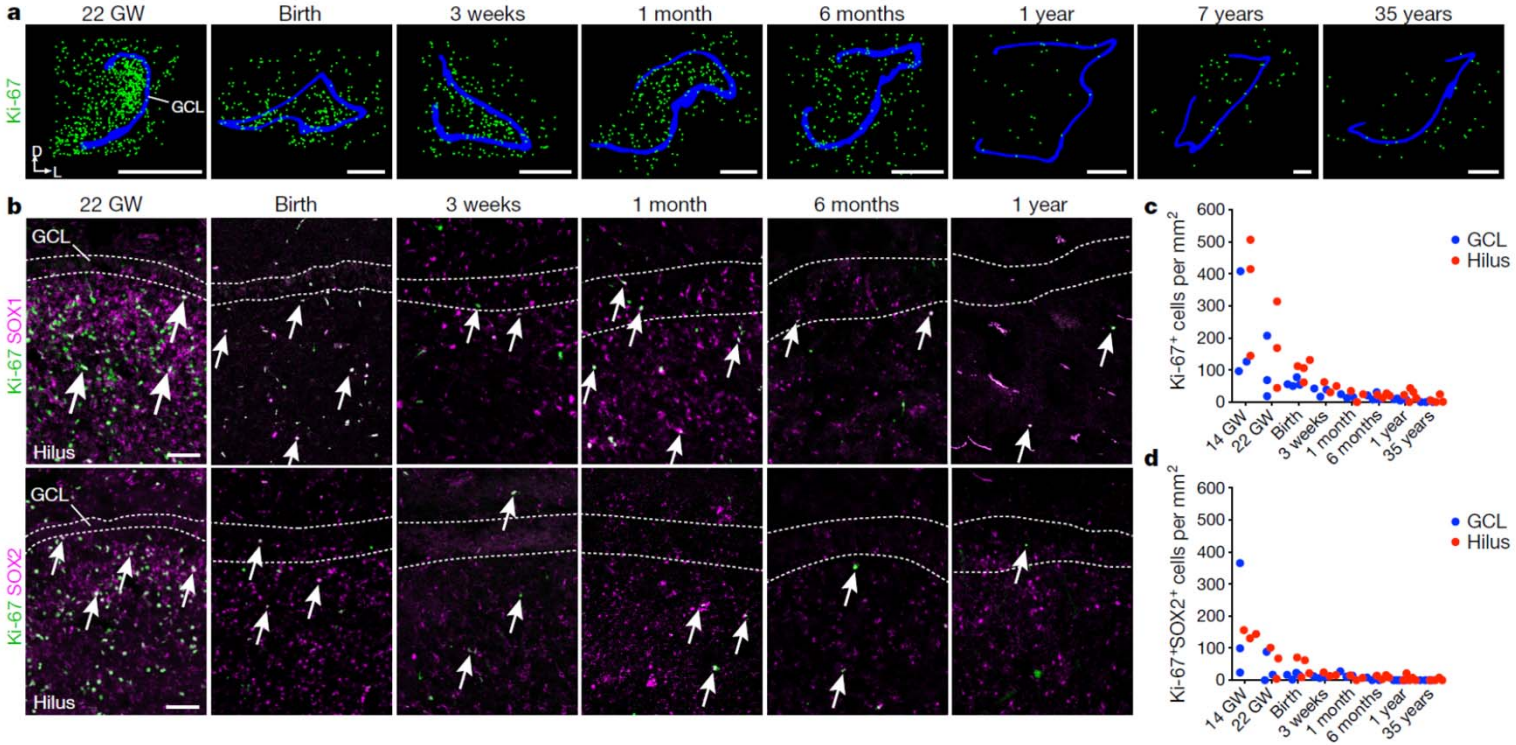


Figure 2 | Human DG proliferation declines sharply during infancy and a layer of proliferating progenitors does not form in the SGZ. **a**, Maps of Ki-67$^+$ (green) cells in the DG from samples of individuals that were between 22 gestational weeks and 35 years of age; GCL in blue. **b**, Ki-67$^+$SOX1$^+$ and Ki-67$^+$SOX2$^+$ cells (arrows) are distributed across the hilus and GCL and the number of double-positive cells decreases between 22 gestational weeks and 1 year of age. **c**, **d**, Quantification of Ki-67$^+$ (**c**) and Ki-67$^+$SOX2$^+$ (**d**) cells in the hilus and GCL. For quantifications, dots indicate staining replicates ($\geq$3) (each age $n = 1$). Scale bars, 1 mm (**a**) and 100 µm (**b**).

- Sorrells SF, [illegible] neurogenesis drops sharply in children to undetectable levels in adults. Nature. 2018;555(7696):377-381.

V References

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Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature. 2018;555(7696):377-81

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